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Determination of zopiclone enantiomers in plasma by liquid chromatography using a chiral cellulose carbamate column

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ABSTRACT

The enantiomers of zopiclone were determined in human plasma using a sequential achiral–chiral liquid chromatographic method. Zopiclone was separated from the biological matrix and quantified on an achiral silica column. The limit of detection was 5 ng/ml. The eluent fraction containing zopiclone was collected, evaporated, reconstituted with the mobile phase and injected onto a chiral cellulose carbamate column where the enantiomeric ratio was calculated. This validated method, applied to a pilot study, suggests that pharmacokinetics of zopiclone is stereoselective.

INTRODUCTION

Zopiclone, (+,−)-6-(5-chloro-2-piridyl)-6,7-dihydro-7-oxo-5H-pyrrolo[3,4-*b*]pyrazin-7-one-4-methyl-1-piperazine carboxylate (ester, racemate, I) (Fig. 1), belongs to the cyclopyrrolone class and shows hypnotic activity. It is commercialized as a racemate (*rac*-zop). Liquid chromatographic methods have been described for the determination of zopiclone in human plasma and urine [1–3] and pharmacokinetic studies [2,4,5] have been published but have been performed on the racemic mixture. This paper presents a sequential chiral method to separate

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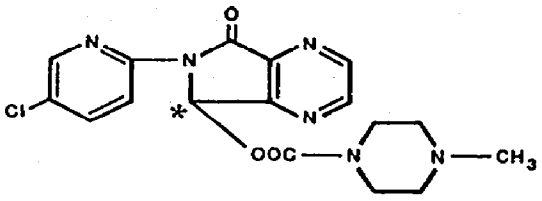
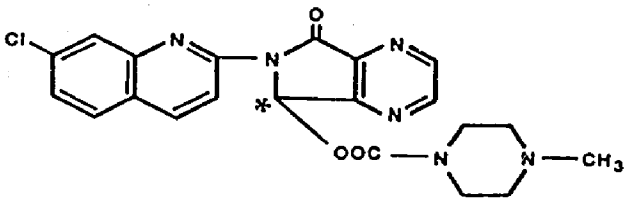


Zopiclone (I)



Internal standard (II)

Fig. 1. Structures of zopiclone (I) and the internal standard (II).

and quantify both isomers in human plasma. Zopiclone was separated from interfering endogenous compounds and quantified on an achiral silica column. The eluents containing zopiclone were collected and injected onto a chiral cellulose-based stationary phase and the enantiomeric ratio was determined.

EXPERIMENTAL

Apparatus

The chromatographic system used for the chiral and achiral methods was composed of a Beckman 114 M pump (Beckman, Gagny, France), a spectrofluorimetric F-535 Shimadzu detector, a Shimadzu C-R6A integrator (Shimadzu, Touzart & Matignon, Vitry, France) and a Rheodyne 7125 injection valve equipped with a 100-μl loop. The temperature of the chiral column was regulated by a Dupont column oven (Dupont Instruments, Orsay, France).

Chemicals

rac-Zop and the internal standard, 6-(7-chloro-2-quinolyl)-5-hydroxy-6,7-dihydro-5H-pyrrolo[3,4-*b*]pyrazin-7-one-4-methyl-1-piperazine carboxylate (ester, II) (Fig. 1), were kindly supplied by Rhône Poulenc Rorer-Théraplix (Paris, France). Ethanol, acetonitrile and methanol (UV grade) were purchased from Merck (Strasbourg, France). Methylene chloride and hexane (UV grade) were purchased from Carlo Erba (Paris La Défence, France). All other chemicals were reagent grade and used as purchased.

Stock solutions

As *rac*-zop and related compounds are not stable in alcohol solutions [1], stock solutions of *rac*-zop (3.78 g/l) and the internal standard (1 g/l) were prepared in acetonitrile.

Analytical chromatography

Achiral chromatography. The column used was a 5-μm Nucleosil silica column (250 mm × 4.6 mm I.D.) (Société Française Chromato Colonne, Neuilly-Plaisance, France). Separation of the internal standard and zopiclone was achieved using a mobile phase composed of acetonitrile–methanol (95:5, v/v). The analyses were carried out at a flow-rate of 1.0 ml/min and at room temperature. Excitation and emission wavelengths were 300 and 470 nm, respectively.

Chiral chromatography. The chiral column used was a cellulose carbamate column (250 mm × 4.6 mm I.D.) (Société Française Chromato Colonne) equipped with a cellulose carbamate guard column (Société Française Chromato Colonne). The separation of zopiclone enantiomers was performed using a mobile phase composed of hexane–ethanol (40:60, v/v). The analyses were performed at a flow-rate of 1.0 ml/min and the temperature was fixed at 35°C. Excitation and emission wavelengths were 300 and 470 nm, respectively.

Extraction procedure

rac-Zop was extracted from plasma according to Le Liboux *et al.* [3]: 100 μl of a solution of internal standard (2 μg/ml), 1 ml of a 0.01 *M* sodium phosphate buffer pH 8 solution and 10 ml of a methylene chloride–2-propanol (90:10, v/v) mixture were added to 1.0 ml of human plasma. The mixture was gently shaken (10 min) and centrifuged (2000 *g*, 10 min). The organic phase was separated and evaporated under nitrogen at 37°C. The remaining solid was reconstituted with 120 μl of the mobile phase, and 100 μl were injected into the achiral column. After separation of *rac*-zop from the biological matrix and detection on the achiral system, fractions of eluent containing *rac*-zop were collected and evaporated at 37°C under nitrogen. The residues were reconstituted with 120 μl of the mobile phase, and 100 μl were injected into the chiral carbamate cellulose column.

Method validation of achiral chromatography

Recovery. The recovery of zopiclone in plasma samples was determined by comparing the peak heights of each sample with the peak heights of standard solutions in mobile phase at each concentration used in the calibration curve. This assay procedure was performed on five days.

Linearity. A standard curve was determined by preparing samples by addition of *rac*-zop to drug-free plasma at the following concentrations: 11.6, 23.3, 50.5, 77.8 and 97.2 ng/ml. This assay was performed on three calibration curves. The samples were analyzed and evaluated by linear least-squares regression.

Precision and reproducibility. Samples were prepared for an inter-day (three days) and intra-day validation. Five samples of the low (11.6 ng/ml) and the high (97.2 ng/ml) concentrations were prepared for the calculation of the coefficient of variation.

Accuracy. The accuracy of the method was determined by injecting samples containing theoretical amounts of zopiclone at the same concentrations as those

used for the calibration curve but spiked by another analyst. Calculated values were compared with theoretical values and the percentage error was determined.

Limit of detection. The limit of detection was determined by analyzing decreasing plasma concentrations of zopiclone and was considered as a signal-to-noise ratio of 3. This assay was performed on three samples.

Method validation of chiral chromatography

During the validation on the achiral chromatographic system, fractions of *rac*-zop were collected and analyzed on the chiral column.

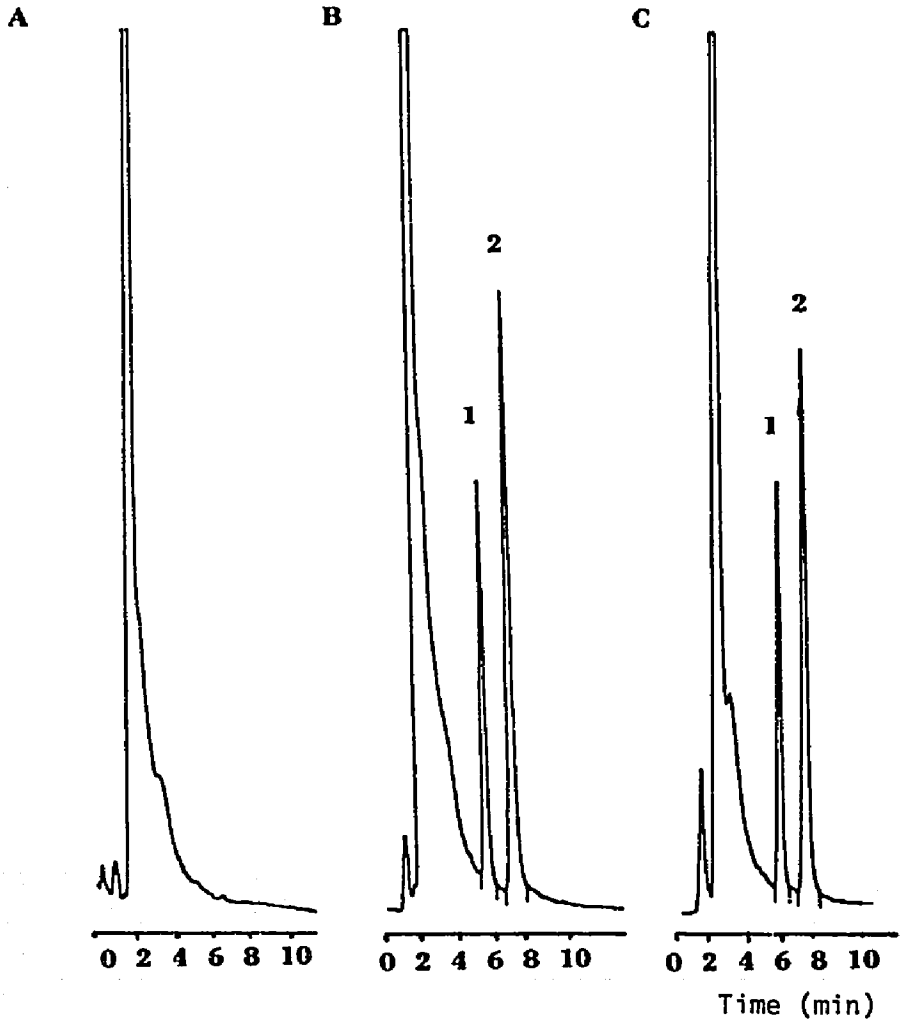


Fig. 2. Separation of extracted plasma samples on a silica column. (A) Blank plasma; (B) plasma spiked with 77.8 ng/ml racemic zopiclone; (C) plasma sample of a volunteer 45 min after drug administration. Peaks: 1 = internal standard; 2 = racemic zopiclone. For chromatographic conditions, see text.

TABLE I

INTRA-DAY AND INTER-DAY VALIDATION OF THE METHOD ON THE ACHIRAL CHROMATOGRAPHIC SYSTEM (PRECISION AND REPRODUCIBILITY)

Sample	Spiked serum concentration (ng/ml)	Mean determined concentration (ng/ml)	Coefficient of variation ($n = 5$) (%)
Intra-day validation			
Low	11.66	14.75	6.00
High	97.20	94.6	8.08
Inter-day validation			
Low	11.66	14.08	6.81
High	97.20	97.86	9.07

RESULTS AND DISCUSSION

Achiral chromatography

Under the chromatographic conditions used in this study, the capacity factors (k') of zopiclone and internal standard were 2.75 and 1.95, respectively. The retention volume (V_0) was determined by injection of acetonitrile. The chromatograms obtained for a blank plasma, a spiked plasma and a patient sample are presented in Fig. 2. The recovery was 92 ± 9%.

The standard curve for zopiclone was linear over the range investigated. Evaluated by linear least-squares regression ($Y = ax + b$), the analysis parameters were as follows: intercept = 0.0509; slope = 0.0175 (S.D. 0.0014); correlation coefficient = 0.9980 ($n = 3$).

The intra-day and inter-day reproducibility and precision are given in Table I. The accuracy of the method is given in Table II. The limit of detection was 5 ng/ml [coefficient of variation (C.V.) 6.9%].

TABLE II

ACCURACY OF THE DETERMINATION OF ZOPICLONE IN PLASMA

Theoretical concentration (ng/ml)	Concentration found (ng/ml)	Error (%)
11.66	12.06	3.4
23.32	21.00	9.8
50.51	53.34	5.6
77.76	76.25	1.9
97.20	97.08	0.1

Chiral chromatography

Chromatographic conditions for the cellulose carbamate column were optimized using different mobile phases composed of hexane and alcohol [6]. Ethanol and 2-propanol gave similar results but ethanol was preferred to 2-propanol because of its lower viscosity. Temperature was then increased to 35°C in order to decrease the capacity factors of both enantiomers. Furthermore, it decreased the

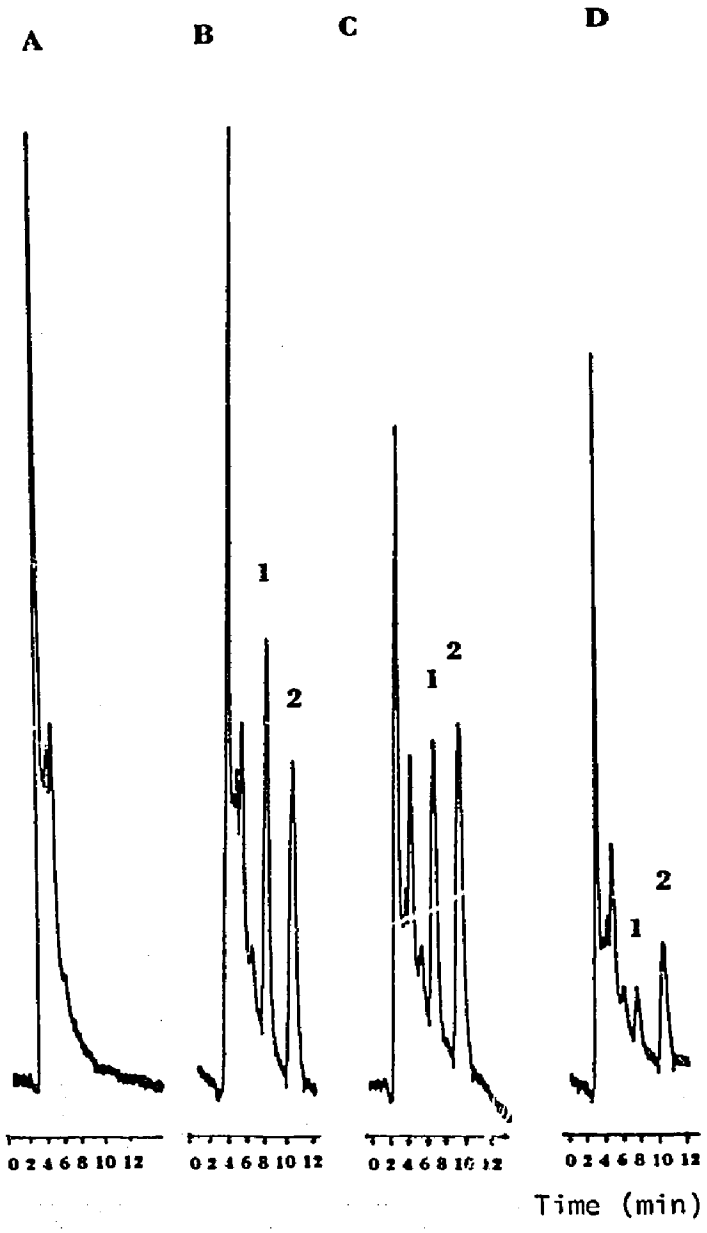


Fig. 3. Separation of zopiclone enantiomers on a cellulose carbamate column (fractions collected from the silica column). (A) Blank plasma; (B) plasma spiked with 77.8 ng/ml racemic zopiclone; (C) plasma sample of a volunteer 45 min after drug administration; (D) plasma sample of a volunteer 6 h after drug administration. Peaks: 1 = (−)zopiclone; 2 = (+)zopiclone. For chromatographic conditions, see text.

viscosity of the mobile phase and the pressure and allowed the use of a higher flow-rate.

The elution order was determined by injecting the separated enantiomers into the chiral column (Rhône Poulenc Rorer Théraplix).

Under the chromatographic conditions used with chiral column, capacity factors of (−)zop and (+)zop were 5.6 and 7.4, respectively, and the selectivity factor was 1.32.

Chromatograms of the fractions collected from a blank plasma, a spiked plasma and a patient sample are presented in Fig. 3.

During the intra-day and the inter-day validation, the enantiomeric ratio (−)zop/(+)zop varied from 0.94 to 1.03.

Pharmacokinetic pilot study

A 15-mg sample of *rac*-zop (Imovane, 7.5 mg, two tablets, Rhône Poulenc Rorer Théraplix) was administered to a male caucasian volunteer. Plasma samples were collected at the following intervals: 0, 30, 45, 60 and 90 min and 2, 4 and 6 h. These samples were extracted and analysed on the achiral and chiral chromatographic systems. The pharmacokinetics of (−)zop and (+)zop are presented in Fig. 4.

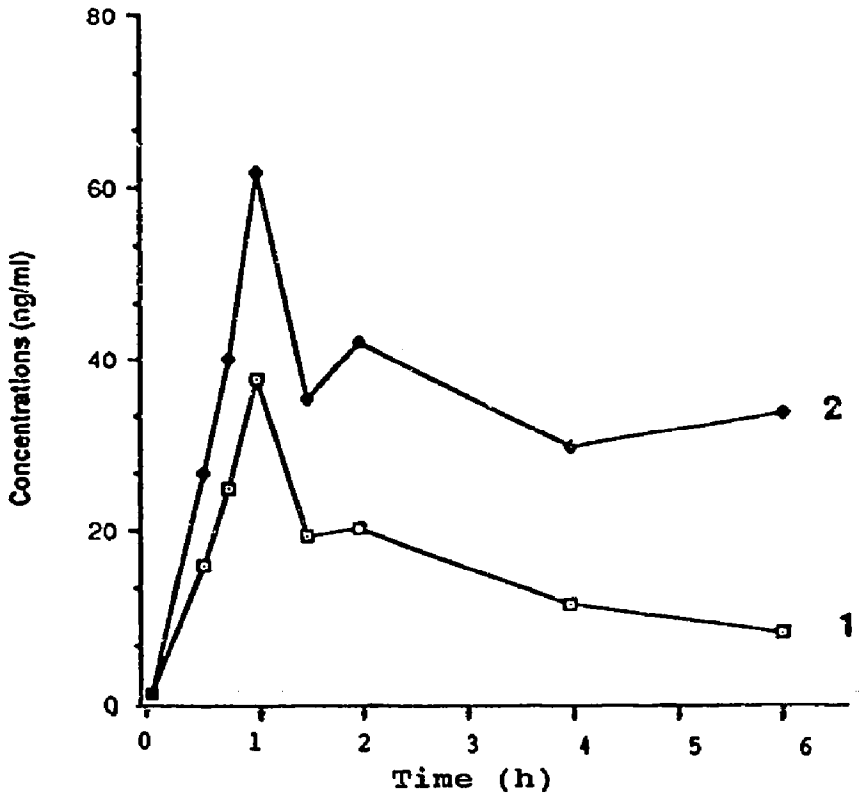


Fig. 4. Plasma concentrations *versus* time curve of (−)zopiclone and (+)zopiclone after administration of 15 mg of racemic zopiclone to a volunteer. 1 = (−)zopiclone; 2 = (+)zopiclone.

The *rac*-zop plasma concentrations obtained in this pilot study are similar to those usually obtained at this dose [2]. These preliminary results show a stereospecificity of the pharmacokinetics of zopiclone. The (−)/(+) enantiomeric ratios varied from 0.59 to 0.25. This difference has yet to be confirmed with a significant number of subjects, and specific pharmacokinetic studies have yet to be performed to determine which steps of the pharmacokinetics (absorption, distribution, metabolism and elimination) are involved.

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